UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/539,229	04/27/2006	John William Chapman	056159-5261	6003

9629 7590 10/08/2010

MORGAN LEWIS & BOCKIUS LLP
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004

EXAMINER	
STEADMAN, DAVID J	

ART UNIT	PAPER NUMBER
1656	

MAIL DATE	DELIVERY MODE
10/08/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/539,229	CHAPMAN ET AL.	
	Examiner	Art Unit	
	David J. Steadman	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 February 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,6,7,12,14-16 and 19* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,6,7,12,14-16 and 19* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/6/10,5/10/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Appendix A*.

DETAILED ACTION

Status of the Application

[1] A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/6/09 has been entered.

[2] Claims 1, 6-7, 12, 14-16, and 19 are pending in the application.

[3] Applicant's amendment to the claims, filed on 2/23/10, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

[4] Receipt of information disclosure statements, filed on 1/6/10 and 5/10/10, is acknowledged.

[5] Applicant's arguments filed on 2/23/10 in response to the Office communication mailed on 12/9/09 are acknowledged. Applicant's arguments have been fully considered and are deemed to be persuasive to overcome at least one of the rejections and/or objections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. Any rejections and/or objections applied to claims 9 and 17 are withdrawn in view of the instant amendment to cancel these claims.

[6] The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Information Disclosure Statement

[7] The information disclosure statements submitted on 1/6/10 and 5/10/10 were filed before the mailing of the instant first Office action on the merits after a request for continued examination. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner. A copy of Forms PTO/SB/08 is attached to the instant Office action

Claim Objection

[8] Claim 1 is newly objected to in the recitation of “a fungal host cell" (lines 2-3) and in the interest of improving claim form, it is suggested that a comma be inserted immediately after the noted phrase.

[9] Claim 1 is newly objected to in the inconsistent use of claim terminology, *i.e.,* “the AFP” and “the type III AFP”. In the interest of improving claim form, it is suggested that claim 1 be amended, *e.g.,* to replace “the AFP” in line 2 with “the type III AFP”.

[10] Claim 19 is newly objected to in the inconsistent use of claim terminology, *i.e.,* “the type III HPLC-12 AFP”, “the expressed AFP”, and “type III AFP”. In the interest of improving claim form, it is suggested that claim 19 be amended, *e.g.,* to replace “AFP” in line 5 and “type III AFP” in line 6 with “type III HPLC-12 AFP”.

Claim Rejections - 35 USC § 112, Second Paragraph

[11] Claim 19 is newly rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 19 is indefinite in the recitation of "…the expressed AFP has increased ice recrystallization…" because it is unclear as to whether this phrase refers to the "type III HPLC-12 AFP" only or to both the "type III HPLC-12" and "functional equivalents thereof".

Claim 19 is also indefinite in the recitation of "functional equivalents thereof having at least 80% amino acid sequence identity with SEQ ID NO:1" because the specification defines "functional equivalent" as "any polypeptide whose sequence has at least 80%...sequence identity with...SEQ ID NO:1" and it is unclear as to whether the scope of recited "functional equivalents" in claim 19 is limited to those that have at least 80% amino acid sequence identity with SEQ ID NO:1, or is intended to encompass functional equivalents of functional equivalents, *i.e.,* those that have at least 80% amino acid sequence identity with polypeptides that are at least 80% amino acid sequence identity with SEQ ID NO:1.

If applicant intends for the "functional equivalents" to be limited to those that have at least 80% amino acid sequence identity with SEQ ID NO:1, recitation of "type III HPLC-12 AFP" in claim 19 is sufficient because the specification's definition of "type III

HPLC-12 AFP" already encompasses those polypeptides that have at least 80% amino acid sequence identity with SEQ ID NO:1.

It is suggested that applicant clarify the meaning of the claim by, *e.g.,* re-writing claim 19 as follows: "A method for producing a type III HPLC-12 antifreeze protein (AFP), comprising expressing a nucleic acid sequence encoding a type III HPLC-12 AFP in a *Saccharomyces cerevisiae* host cell which is deficient in pmt1 and/or pmt2, and wherein the expressed type III HPLC-12 AFP has increased ice recrystallization inhibitory activity in comparison to glycosylated type III HPLC-12 AFP.

Claim Rejections - 35 USC § 112, First Paragraph

[12] The scope of enablement rejection of claims 1, 6-7, 12, and 14-16 under 35 U.S.C. 112, first paragraph, is maintained. Newly added claim 19 is included in the rejection for reasons that follow. As such, claims 1, 6-7, 12, 14-16, and 19 are rejected herein because the specification, while being enabling for a method for producing a type III HPLC-12 antifreeze protein (AFP) peptide in a *Saccharomyces cerevisiae* cell modified to delete the protein mannosyl transferase 1 (pmt1) and/or pmt2 genes, wherein the type III HPLC-12 AFP peptide has increased ice recrystallization inhibitory activity as compared to a glycosylated type III HPLC-12 AFP, does not reasonably provide enablement for methods using all *S. cerevisiae* cells that are deficient in pmt1 polypeptide and/or pmt2 polypeptide as encompassed by claims 1 and 19 and methods for producing all "functional equivalents" of a type III HPLC-12 AFP as encompassed by claim 19. The specification does not enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

"The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below.

The nature of the invention: According to specification, type III HPLC-12 AFP can be recombinantly produced using a yeast host cell (p. 2, lines 23-30), however, type III HPLC-12 AFP produced in yeast has lower recrystallization inhibition activity relative to the protein isolated from its natural source, *i.e.,* Ocean Pout blood (paragraph bridging pp. 3-4). The reduced activity is due to O-glycosylation of the protein by the yeast, which does not occur in Ocean Pout (p. 4, lines 1-4). The nature of the invention is the use of a fungal host cell that is deficient in protein glycosylation, including a *S. cerevisiae* that is deficient in pmt1 polypeptide and/or pmt2 polypeptide (p. 5, lines 5-21).

The breadth of the claims: Claims 1 (claims 14-16 dependent therefrom), 6-7, 12, and 19 are drawn to a method for producing an AFP protein using a mutant *S. cerevisiae* cell that is deficient in a protein mannosyl transferase 1 (pmt1) polypeptide and/or a protein mannosyl transferase 2 (pmt2) polypeptide.

The recited *S. cerevisiae* mutant cell that is deficient in pmt1 polypeptide and/or pmt2 polypeptide encompasses an *S. cerevisiae* cell having *any* modification to the cell that results in a deficiency in pmt1 polypeptide and/or pmt2 polypeptide, including, *e.g.,* modification to alter the activity of a protein or proteins that regulate pmt1 and/or pmt2 expression and/or modification to the pmt1 and/or pmt2 mRNA or protein to facilitate degradation.

Claim 19 is drawn to a method for producing a type III HPLC-12 AFP and *functional equivalents*…wherein the expressed AFP has increased ice recrystallization inhibitory activity" (emphasis added). A "function" of a type III HPLC-12 polypeptide is eliciting an antibody, which is shared by essentially all polypeptides of a sufficient length. Since claim 19 refers only to the "expressed AFP" as having increased ice recrystallization inhibitory activity – not the "functional equivalents" – the functional equivalents includes polypeptides that do not have ice recrystallization inhibitory activity.

The enablement provided by the specification is not commensurate in scope with the claims with regard to the *S. cerevisiae* mutant cells used in the methods of claims 1 and 19 and the "functional equivalents" produced in the method of claim 19.

The state of the prior art; The level of one of ordinary skill; and The level of predictability in the art: At the time of the invention, the recombinant production of type

III HPLC-12 AFP using a *S. cerevisiae* host cell was well known in the art as shown by, *e.g.,* Chapman et al. (WO 97/02343; cited in the IDS filed on 6/16/05) at pp. 35-37.

Also, at the time of the invention, the reference of Ng et al. (US Patent Application Publication 2002/0068325; cited in Form PTO-892 mailed on 1/22/08) teaches "it is possible that most heterologous proteins can become O-linked glycosylated" when produced in yeast and that O-linked glycosylation can result in misfolding and compromise activity and stability (p. 6, paragraph 69). To avoid O-linked glycosylation of a recombinantly produced protein, Ng et al. teaches the use of a *S. cerevisiae* yeast strain modified to delete pmt genes.

The references of Gentzsch et al. (*FEBS Lett.* 377:128-130, 1995; cited in the PTO-892 mailed on 1/22/08) and Gentzsch et al. (*Glycobiology* 7:481-486, 1997) show that making pmt1- and/or pmt2-deleted strains of *S. cerevisiae* and using these stains for heterologous protein expression was routine experimentation at the time of the invention.

However, other than deleting pmt1 and/or pmt2 in a *S. cerevisiae* host cell, the prior art does not appear to disclose other methods for generating a strain of *S. cerevisiae* that is deficient in pmt1 and/or pmt2 polypeptide.

Also, with respect to the unpredictability of a variant polypeptide maintaining activity, MPEP 2144.08.II.A.4.(c) states, "[i]n the area of biotechnology, an exemplified species may differ from a claimed species by a conservative substitution ("the replacement in a protein of one amino acid by another, chemically similar, amino acid... [which] is generally expected to lead to either no change or only a small change in the

properties of the protein." Dictionary of Biochemistry and Molecular Biology 97 (John Wiley & Sons, 2d ed. 1989)). The effect of a conservative substitution on protein function depends on the nature of the substitution and its location in the chain. Although at some locations a conservative substitution may be benign, in some proteins only one amino acid is allowed at a given position. For example, the gain or loss of even one methyl group can destabilize the structure if close packing is required in the interior of domains. James Darnell et al., Molecular Cell Biology 51 (2d ed. 1990)." As such, the prior art acknowledges that even a single *conservative* amino acid substitution may alter protein activity.

The amount of direction provided by the inventor and The existence of working examples: The specification discloses only a single working examples of the claimed method, *i.e.,* a method for recombinantly producing an type III AFP HPLC-12 peptide in a *Saccharomyces cerevisiae* host with deleted pmt1 and/or pmt2 genes. The specification fails to disclose any guidance regarding modification(s) to *S. cerevisiae* that result in a deficiency of pmt1 and/or pmt2 polypeptide. Moreover, the specification fails to disclose any guidance regarding modification to a type III HPLC-12 AFP with an expectation that the variant will maintain ice recrystallization inhibitory activity and fails to disclose guidance for using those variants that do not.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: While recombinant protein expression of heterologous proteins using an *S. cerevisiae* with deleted pmt1 and/or pmt2 genes was well-known in the prior art at the time of the invention, it was not routine to modify an *S. cerevisiae* to

achieve deficiency of pmt1 and/or pmt2 protein by any method or to use a variant of a type III HPLC-12 AFP protein without ice recrystallization inhibitory activity as broadly encompassed by the claims.

In view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, the high level of unpredictability as evidenced by the prior art, and the amount of experimentation required, undue experimentation is necessary for a skilled artisan to make and use the entire scope of the claimed invention. Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

RESPONSE TO REMARKS: Beginning at p. 4 of the instant remarks, applicant argues the specification and prior art fully enable all methods of achieving pmt1 and/or pmt2 deficiency in an *S. cerevisiae* host cell.

Applicant's argument is not found persuasive. In view of the detailed analysis of the Factors of *In re Wands,* set forth above, the examiner maintains that the specification and prior art fail to enable the full scope of the claims, particularly with

respect to the scope of mutant *S. cerevisiae* cells that are deficient in pmt1 and/or pmt2 as broadly encompassed by the claims. Applicant's alleged methods of down-regulating pmt1 and/or a pmt2 gene expression (instant remarks at p. 5) are acknowledged, however, the modifications to achieve pmt1 and/or pmt2 deficiency in an *S. cerevisiae* host cell are not so limited and, as noted above, broadly encompass, *e.g.,* modification to alter the activity of a protein or proteins that regulate pmt1 and/or pmt2 expression and modification to the pmt1 and/or pmt2 mRNA or protein to facilitate degradation.

[13] The written description rejection of claims 1, 6-7, 12, and 14-16 under 35 U.S.C. 112, first paragraph, is maintained. Newly added claim 19 is included in the rejection for reasons that follow. As such, claims 1, 6-7, 12, 14-16, and 19 are rejected herein.

Claims 1 (claims 14-16 dependent therefrom), 6-7, 12, and 19 are drawn to a method for producing an AFP protein using a genus of mutant *Saccharomyces cerevisiae* cells that are deficient in a protein mannosyl transferase 1 (pmt1) polypeptide and/or a protein mannosyl transferase 2 (pmt2) polypeptide.

For claims drawn to a genus, MPEP § 2163 states the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such

identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

In this case, the specification discloses only a single representative species of the recited genus of *S. cerevisiae* cells that are deficient in pmt1 and/or pmt2, *i.e.*, a *S. cerevisiae* cell modified to delete the pmt1 and/or pmt2 genes. The specification fails to describe any additional representative species of the recited genus of *S. cerevisiae* cells that are deficient in pmt1 and/or pmt2. While MPEP § 2163 acknowledges that in certain situations "one species adequately supports a genus," it also acknowledges that "[f]or inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus." In the instant case, the recited genus of *S. cerevisiae* cells that are deficient in pmt1 polypeptide and/or pmt2 polypeptide encompasses species that are widely variant, including an *S. cerevisiae* cell that is deficient in pmt1 polypeptide and/or pmt2 polypeptide by *any* modification to the cell, including, *e.g.,* modification to alter the activity of a protein or proteins that regulate pmt1 and/or pmt2 expression; modification to the *pmt1* and/or *pmt2* gene promoter and/or transcriptional elements; and modification to the pmt1 and/or pmt2 mRNA or protein to facilitate degradation. In this case, the disclosure of the single representative species of *S. cerevisiae* cells that are

deficient in pmt1 polypeptide and/or pmt2 polypeptide is insufficient to be representative of the attributes and features of all species as encompassed by the claims.

Given the lack of description of a representative number of polypeptides, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicant was in possession of the claimed invention.

RESPONSE TO REMARKS: The instant remarks do not appear to address the written description rejection.

Claim Rejections - 35 USC § 103

[14] Claim(s) 1, 6-7, 12, and 14-16 under 35 U.S.C. 103(a) as being unpatentable over Chapman et al. (WO 97/02343; cited in the IDS filed on 6/16/05; "Chapman") in view of Ng et al. (US Patent Application Publication 2002/0068325; cited in the PTO-892 mailed on 1/22/08; "Ng") and Gentzsch et al. (*FEBS Lett.* 377:128-130, 1995; cited in the PTO-892 mailed on 1/22/08; "Gentzsch") is maintained. Newly added claim 19 is included in the rejection for reasons that follow. As such, claims 1, 6-7, 12, 14-16, and 19 are rejected herein.

Chapman teaches a method for recombinantly producing an Ocean Pout type III HPLC-12 AFP peptide in a *Saccharomyces cerevisiae* host transfected with a vector encoding said peptide. See Example 5 at pp. 35-37. The type III HPLC-12 AFP

polypeptide of Chapman is 100% identical to SEQ ID NO:1 herein (see Appendix A sequence alignment).

Chapman does not teach or suggest the use of a pmt1- or pmt2-deficient strain of *S. cerevisiae* for use in the disclosed method.

Motivation for using a mutant strain of *S. cerevisiae* modified to delete pmt1- and/or pmt2 genes is taught by Ng. The reference of Ng teaches "it is possible that most heterologous proteins can become O-linked glycosylated" when produced in yeast and that O-linked glycosylation can result in misfolding and compromise activity or stability (p. 6, paragraph 69). Ng discloses O-linked glycosylation in yeast is due to a family of genes called protein mannosyltransferases (PMT) (paragraph 68 at p. 6). Ng teaches that since there are 6 PMT genes in yeast that are non-redundant and exhibit differences in substrate specificity, deletion strains of any of the six genes may provide the needed inhibition of aberrant O-glycosylation (p. 6, paragraph 71), specifically disclosing a comparison of protein expression using wild-type, pmt1, and pmt2 mutant cells (p. 4, paragraph 57). According to Ng, using pmt-deleted *S. cerevisiae* as an expression host improves yields and activity of a heterologously expressed protein (p. 1, paragraph 12) and the use of such strains is a solution for overcoming a problem that has limited the potential of low cost expression of commercially important molecules in yeast (paragraph 71 at p. 6). In order to determine the potential effects of O-linked glycosylation, Ng discloses a comparison of heterologous protein expression using a wild-type, pmt1 and pmt2 deletion strain of *S. cerevisiae* (p. 4, paragraph 57), where O-

glycosylation of the heterologous protein is dependent on pmt1 and pmt2 (p. 12, paragraph 152, middle to bottom).

Gentzsch teaches pmt1 and pmt2 deletion mutants of *S. cerevisiae* (p. 28, column 2, paragraph 2.1); teaches the polypeptides encoded by pmt1 and pmt2 function as a heterodimer having O-mannosyltransferase activity in the O-glycosylation of polypeptides (p. 128, abstract); and teaches that disruption of each of these genes leads to “a dramatic decrease of mannosyltransferase activity in vitro” (p. 128, abstract).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Chapman, Ng, and Gentzsch to compare type III HPLC-12 expression using wild-type and pmt1- and/or pmt2-deleted *S. cerevisiae.* One would have been motivated to do this in order to determine if the type III HPLC-12 expressed using pmt1- and/or pmt2-deleted *S. cerevisiae* has increased activity and/or stability as suggested by Ng. One would have had a reasonable expectation of success to compare type III HPLC-12 expression using wild-type and pmt1- and/or pmt2-deleted *S. cerevisiae* because of the results of Chapman, Ng, and Gentzsch. Therefore, the method of claims 1, 6-7, 12, 14-16, and 19 would have been obvious to one of ordinary skill in the art at the time of the invention.

RESPONSE TO REMARKS: At pp. 5-7 of the instant remarks, applicant argues the Office has not provided evidence or prior art that discloses: how to predict which pmt enzymes will glycosylate a particular protein; how to predict whether glycosylation has a positive or negative effect; or how to predict that a yeast-expressed protein's

function would be improved with less glycosylation. Applicant refers to the reference of Gentzsch et al. (*Glycobiology* 7:481-486, 1997) as supporting the position that there is no way to predict which pmt enzymes will glycosylate a particular protein.

Applicant's argument is not found persuasive. Initially, it is noted that the examiner can find no copy of the reference of Gentzsch (*Glycobiology* 7:481-486, 1997) in the application file, however, in the interest of advancing prosecution, a copy of this reference has been made of record. Contrary to applicant's position, *a priori* knowledge of which *S. cerevisiae* pmt's – if any – glycosylate a polypeptide or the effects of pmt glycosylation on a polypeptide's function are not required to compare type III HPLC-12 expression using wild-type and pmt1- and/or pmt2-deleted *S. cerevisiae.* That one could not predict which *S. cerevisiae* pmt's – if any – glycosylate a polypeptide or the effects of pmt glycosylation on a polypeptide's function further supports the motivation to compare type III HPLC-12 expression using wild-type and pmt-deleted *S. cerevisiae.* The point of the comparison of Ng is to ascertain knowledge regarding which *S. cerevisiae* pmt's – if any – glycosylate a polypeptide or the effects of pmt glycosylation on a polypeptide's function. In view of Ng's disclosed potential advantages of using a pmt-deleted strain of *S. cerevisiae* for recombinant protein expression, one would have been motivated to compare type III HPLC-12 expression using wild-type and pmt1- and/or pmt2-deleted *S. cerevisiae.* Moreover, applicant's cited reference of Gentzsch uses such a comparison and provides further evidence that only routine experimentation is required to make pmt-deleted strains of *S. cerevisiae* at the time of the invention.

At pp. 6-7 of the instant remarks applicant further argues glycosylation has no effect on the activity of AFP expressed in rye grass, referring to an attached reference of Pudney (*Archives Biochem. Biophys.* 410:238-245, 2003), and one of skill in the art would have recognized that glycosylation of AFP is not significant to AFP's production and function.

Applicant's argument is not found persuasive. Initially, it is noted that the examiner can find no copy of Pudney (*Archives Biochem. Biophys.* 410:238-245, 2003) in the application file, however, in the interest of advancing prosecution, a copy of this reference has been made of record. It is further noted that the publication date of the reference of is after the time of the invention, which is 12/20/02, and would not have been available to one of ordinary skill in the art. Moreover, the reference of Pudney is related to characterization of an AFP from rye grass, expressed in *P. pastoris* and *E. coli* and is not related to type III HPLC-12 AFP expression in *S. cerevisiae.*

At p. 7 of the instant remarks, applicant refers to the reference of Sanders (*J. Cell Biol.* 145:1177-1188, 1999), which allegedly discloses that glycosylation is required for the stability and function of a protein produced in yeast.

Applicant's argument is not found persuasive. Initially, it is noted that the examiner can find no copy of Sanders (*J. Cell Biol.* 145:1177-1188, 1999) in the application file, however, in the interest of advancing prosecution, a copy of this reference has been made of record. The reference of Sanders is related to pmt4 glycosylation of a protein referred to as "Axl2/Bud10p", which is described as a type I integral membrane protein that is thought to provide spatial information for the axial

pattern. As with Pudney, Sanders is not related to type III HPLC-12 AFP expression in *S. cerevisiae.*

At p. 7 of the instant remarks, applicant argues one would have dismissed the Ng reference because: 1) Ng discloses that modulating glycosylation is useful if the protein is experiencing mis-folding in yeast and Chapman demonstrates that this was not the case; and 2) it was known that modulating glycosylation exerted unpredictable effects.

Applicant's argument is not found persuasive. Contrary to applicant's assertion, Chapman only indicates that *S. cerevisiae*-expressed type III HPLC-12 is *functional* – there is no indication in Chapman that the type III HPLC-12 protein is not at least in part misfolded. Ng recognizes that "it is possible that most heterologous proteins can become O-linked glycosylated" when produced in yeast and that O-linked glycosylation can result in misfolding and compromise activity or stability (p. 6, paragraph 69). As acknowledged by applicant, there was no way to predict whether a heterologous protein expressed in *S. cerevisiae* is glycosylated or the resulting functional effects of such glycosylation and as such, one would have been motivated to compare type III HPLC-12 expression using wild-type and pmt1- and/or pmt2-deleted *S. cerevisiae* to determine if expression using pmt1- and/or pmt2-deleted *S. cerevisiae* resulted in a protein with improved activity or stability as suggested by Ng. Even assuming *arguendo* the reference of Chapman provided evidence that each and every molecule of *S. cerevisiae*-expressed type III HPLC-12 is properly folded, Ng provides an additional advantage of heterologous expression using pmt-deleted *S. cerevisiae,* which is improvement of yield (p. 1, paragraph 12).

In view of Ng's disclosed potential advantages of using a pmt-deleted strain of *S. cerevisiae* for recombinant protein expression, one would have been motivated to compare type III HPLC-12 expression using wild-type and pmt1- and/or pmt2-deleted *S. cerevisiae.* Applicant has presented no compelling reason to the contrary. At least for the reasons of record and the reasons set forth above, the examiner maintains that the method of claims 1, 6-7, 12, 14-16, and 19 would have been obvious to one of ordinary skill in the art at the time of the invention.

Claim Rejections – Double Patenting

[15] The rejection of claims 1, 6-7, 12, and 14-16 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-9 of US Patent 7,297,516 ("'516 patent") in view of Ng (*supra*) and Gentzsch (*supra*) is maintained. Newly added claim 19 is included in the rejection for reasons that follow. As such, claims 1, 6-7, 12, 14-16, and 19 are rejected herein.

An obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but an examined application claim is not patentably distinct from the reference claim(s) because the examined claim is either anticipated by, or would have been obvious over, the reference claim(s). See *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); and *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985). Although the conflicting claims are not identical, they are not patentably distinct from each other. The difference between claims 1, 6-7, 12, 14-16, and 19 and

the claims of the '516 patent is that method of the claims of the '516 patent do not require the use of a pmt1 and/or pmt2-deficient yeast for protein production.

However, motivation to use a pmt1 and/or pmt2-deficient yeast for protein production is provided by the reference of Ng (*supra*) as noted above.

Gentzsch teaches pmt1 and pmt2 deletion mutants of *S. cerevisiae* (p. 28, column 2, paragraph 2.1); teaches the polypeptides encoded by pmt1 and pmt2 function as a heterodimer having O-mannosyltransferase activity in the O-glycosylation of polypeptides (p. 128, abstract); and teaches that disruption of each of these genes leads to "a dramatic decrease of mannosyltransferase activity in vitro" (p. 128, abstract).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of the '516 patent to use a pmt1- and/or a pmt2-deficient strain of *S. cerevisiae*. One would have been motivated to do this because of the potential advantages of using such a strain as noted by Ng above.

[16] Claims 1, 6-7, 12, 14-16, and 19 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 11 of US Patent 7,560,249 (hereafter "'249 patent") in view of Ng (*supra*) and Gentzsch (*supra*).

Although the conflicting claims are not identical, they are not patentably distinct from each other. The difference between claims 1, 6-7, 12, 14-16, and 19 and the claim of the '249 patent is that method of the claim of the '249 patent does not require the use of a pmt1 and/or pmt2-deficient *S. cerevisiae* for protein production and the heterologous protein is not limited to type III HPLC-12.

However, motivation to use a pmt1 and/or pmt2-deficient yeast for protein production is provided by the reference of Ng (*supra*) as noted above.

Gentzsch teaches pmt1 and pmt2 deletion mutants of *S. cerevisiae* (p. 28, column 2, paragraph 2.1); teaches the polypeptides encoded by pmt1 and pmt2 function as a heterodimer having O-mannosyltransferase activity in the O-glycosylation of polypeptides (p. 128, abstract); and teaches that disruption of each of these genes leads to "a dramatic decrease of mannosyltransferase activity in vitro" (p. 128, abstract).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of the '249 patent to use a pmt1- and/or a pmt2-deficient strain of *S. cerevisiae*. One would have been motivated to do this because of the potential advantages of using such a strain as noted by Ng above.

Furthermore, claims 1, 6-7, 12, 14-16, and 19 cannot be considered patentably distinct over the claim of the '249 patent when there is a specifically disclosed embodiment in the '249 patent that supports the claims of that application and falls within the scope of claims 1, 6-7, 12, 14-16, and 19 herein because it would have been obvious to one having ordinary skill in the art to modify the method of the claim of the '249 patent by selecting a specifically disclosed embodiment that supports the claims, i.e., type III HPLC-12 as the recombinant polypeptide as disclosed at column 7, lines 46-47. One having ordinary skill in the art would have been motivated to do this because that embodiment is disclosed as being a preferred embodiment within the claims of the '249 patent.

RESPONSE TO REMARKS: At pp. 7-8 of the instant remarks, applicant argues the rejection cannot be maintained in view of alleged deficiencies of Chapman, Ng, and Gentzsch.

Applicant's argument is not found persuasive. At least for the reasons set forth above, the use of a pmt1 and/or pmt2 deletion strain of *S. cerevisiae* in the methods of the '516 and '249 patents would have been obvious to one of ordinary skill in the art.

Conclusion

[17] Status of the claims:

- Claims 1, 6-7, 12, 14-16, and 19 are pending.
- Claims 1, 6-7, 12, 14-16, and 19 are rejected.
- No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is 571-272-0942. The examiner can normally be reached on Mon to Fri, 7:30 am to 4:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/David J. Steadman/
Primary Examiner, Art Unit 1656

APPENDIX A

```
AAW11268
ID   AAW11268 standard; peptide; 94 AA.
XX
AC   AAW11268;
XX
DT   27-OCT-1997  (first entry)
XX
DE   Type III AFP variant HPLC-12.
XX
KW   Anti-freeze protein; AFP; HPLC-12; type III AFP; additive; frozen dough;
KW   ice crystal growth; inhibitor; ice recrystallisation; Ocean Pout; WFAFP;
KW   Winter Flounder; food preparation; ice cream.
XX
OS   Synthetic.
XX
PN   WO9702343-A1.
XX
PD   23-JAN-1997.
XX
PF   01-JUL-1996;   96WO-EP002936.
XX
PR   05-JUL-1995;   95EP-00201842.
PR   10-OCT-1995;   95EP-00202732.
XX
PA   (UNIL ) UNILEVER PLC.
PA   (UNIL ) UNILEVER NV.
XX
PI   Chapman JW,  Musters W,  Van Wassenaar PD;
XX
DR   WPI; 1997-108956/10.
DR   N-PSDB; AAT51173.
XX
PT   New type III antifreeze peptide - used for minimising freezing damage to
PT   foods and biological material.
XX
PS   Example 5; Fig 17; 73pp; English.
XX
CC   This sequence represents the HPLC-12 variant of the type III antifreeze
CC   peptide (AFP). The DNA encoding this sequence was constructed with codon
CC   usage optimised for expression in Saccharomyces cerevisiae. This
CC   sequence, and variants of it, can be used as the additives of the
CC   invention. The additives are used to improve properties of a product,
CC   i.e. to modify ice crystal growth so that the size and shape of ice
CC   (especially in regrowth) is altered, minimising potential freezing
damage
CC   by preventing or inhibiting ice recrystallisation on freezing. A similar
CC   effect is achieved by adding to the product (or an ingredient of it) a
CC   host organism that expresses the encoded protein. In yeast, AFP and
HPLC-
CC   12 can be expressed and secreted as an active monomer (suitable for
large
```

```
CC   scale production) having the high AFP activity of a mixture of peptides
CC   isolated from the blood of the Ocean Pout, i.e. almost double the
CC   activity of Winter Flounder AFP (WFAFP). The recombinant host cells
allow
CC   the protein to be formed during food preparation, eliminating the need
CC   for a separate additional step and protein purification. This protein is
CC   used in the same way as other AFP but has higher activity, especially
CC   antifreeze activity, than the same amount of WFAFP. The method is
applied
CC   to foods and biological materials, especially ice cream and frozen dough
CC   or bakery goods. Also contemplated is development of transgenic plants,
CC   fruit, vegetables and animals with improved freezing properties
XX
SQ   Sequence 94 AA;

  Query Match             100.0%;  Score 328;  DB 1;  Length 94;
  Best Local Similarity   100.0%;
  Matches   66;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps
0;

Qy          1 NQASVVANQLIPINTALTLVMMRSEVVTPVGIPAEDIPRLVSMQVNRAVPLGTTLMPDMV 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         29 NQASVVANQLIPINTALTLVMMRSEVVTPVGIPAEDIPRLVSMQVNRAVPLGTTLMPDMV 88

Qy         61 KGYPPA 66
              ||||||
Db         89 KGYPPA 94
```